47. (Amended) The method of Claim 45, wherein the antigens of the transcription units [represent] are from an immunodeficiency virus at different phases of infection [of the immunodeficiency virus].

G2 cont'd

48. (Amended) The method of Claim 45, wherein the antigens of the transcription units [represent] are from immunodeficiency viruses having different tissue tropisms [of the immunodeficiency virus].

49. (Amended) The method of Claim 45, wherein the antigens of the transcription units [represent] are from immunodeficiency viruses having different routes of transmission [of the immunodeficiency virus].

G3

72. (Amended) The composition of Claim 69, wherein each DNA transcription unit comprises DNA encoding an antigen of Env protein from an immunodeficiency virus having a different route of transmission [of human immunodeficiency virus].

REMARKS

The Specification has been amended to correct references to Figures 16A and 16B. The related text refers to plasmids encoding SIV239 proviral DNA and a pCMV/SIV239.dpol insert, which are shown in Figures 16A and 16B. Claim 44 has been amended to use the term "mammal" consistently. No new matter has been added.

Claims 44-51, 62-64, 67-72, 74 and 78-89 are pending.

For the Examiner's convenience, the remainder of this Amendment is set forth under appropriate headings.

Applicants' Invention

The claimed invention is drawn to methods of immunizing a mammal against an immunodeficiency virus, by administering a DNA transcription unit which is expressed in cells of the mammal, eliciting an immune response, whereby the mammal is protected from disease caused by the immunodeficiency virus (Claims 44-51 and 81-89). The invention is also drawn to compositions comprising certain DNA transcription units (Claims 62-64, 67-72 and 74), as well as to plasmid vectors comprising certain constructs (Claims 78-80).

Rejection of Claims under 35 U.S.C. 112, first paragraph

The Examiner maintained the rejection of Claims 44-51, 62-64, 67-72, 74 and 78-89, in that the constructs used in the Declaration filed 2/28/96 (referred to herein as the "Declaration") were different from those described in the Specification, and that the data in the Declaration did not demonstrate protectivity. These issues are discussed in turn below.

Constructs of the Declaration and of the Specification

Certain of the constructs described in the Declaration are based on the SIV proviral DNA and SIV239.dpol insert, shown in the figures at page 39 of the Appendix to the Declaration. These figures correspond exactly to Figures 16A and 16B of the current application. The SIV239.dpol insert described both in the Declaration and in the Specification was generated from two separate plasmids representing the 5'(p239SpSp5') and 3'(p239SpE3') halves of a 239 provirus (page 1 of the Appendix to the Declaration; page 53, lines 21-26 of the Specification). Other constructs are JW4303 based vectors, described at pages 2 and 3 of the Appendix of the Declaration, and at page 54, line 8 *et seq.* of the Specification. As described both in the Appendix to the Declaration and the Specification, these constructs were constructed by cloning polymerase chain reaction

(PCR) amplified fragments of the SIV env sequences shown in the figures at page 39 of the Appendix to the Declaration and also in Figures 16A and 16B of the application.

The constructs described in the Appendix to the Declaration (see, e.g., the Figure on page 39) use PCR inserts that are close, if not identical, in size to those described in the Specification and shown in Figure 17D. For example, a comparison of two of the constructs is set forth below:

Appendix to Declaration	*Specification*
SIV239.sgp110 (1.5 kB insert)	SIV239.sgp120 (1.6 kB insert)
SIV239.sgp130 (2.1 kB insert)	SIV239.sgp140 (2.1 kB insert).

Both the Appendix and the Specification also describe an SIV251.sgp construct (Appendix, SIV251.sgp130; Specification, SIV251.sgp140); and an SIV316.sgp construct (Appendix, SIV316.sgp130; Specification, SIV316.sgp140). It can be seen that the sgp110 constructs in the Appendix to the Declaration correspond to the sgp120 constructs in the Specification, and the sgp130 constructs in the Appendix correspond to the sgp140 constructs in the Specification. The sgp number ("110" or "120", "130" or "140" refers to size of the protein expressed by the construct, when the proteins are examined by polyacrylamide gel electrophoresis (PAGE) (such as that described on page 3 and also on page 11, first full paragraph, of the Appendix to the Declaration), rather than to any specific content of the constructs. See, for example, Table 1 in the Appendix of the Declaration, where the constructs are referred to as "239.sgp120," "239.sgp140," "251.spg [*sic, sgp*]140" and "316.sgp140." One of ordinary skill in the art would recognize that slight variations in size, such as that produced by glycosylation of the proteins, would account for the different sizes of the proteins. However, as shown by the use of the same names for the DNA constructs in Table 1 of the Appendix to the Declaration, and in the Specification, the vectors and their contents (PCR amplified fragments of the SIV env sequences shown in the figure on page 39 of the Appendix to the Declaration, and shown

in Figures 16A and 16B) are essentially the same in both the Declaration and the Specification.

Protectivity of the Constructs

As described in the Specification at page 7, lines 5-11, "immunizing" refers to production of an immune response which protects, *partially or totally*, from the manifestations of infection (i.e., disease) caused by the infectious agent, in that the individual immunized will not be infected, or will be infected to a lesser extent, than would occur without immunization. Thus, "protection" does not refer solely to a change in susceptibility to the disease, but rather, refers to the generation of an immune response that lessens or eliminates the infection and its manifestations.

The Declaration describes the generation of such an immune response. As described therein, four macaques in the multiple route group were protected against manifestations of disease throughout the trial, as these animals were free of clinical signs of AIDS at the time of euthanasia. Furthermore, partial protection from the manifestations of infection was also demonstrated by a more rapid reduction of viral loads to chronic levels in immunized animals, in comparison to the rate of reduction in control animals. Rapid reduction of viral load is particularly useful for protecting a population of animals (in contrast to protecting a single animal) by attenuating the acute phase or infection. Attenuation of the acute phase of infection reduces transmission of infection by reducing the window of time in which an individual has a high virus load.

In view of these considerations, Applicants have shown, for the first time, that immunization with DNA constructs can protect against manifestations of infection.

Model for Disease

The Examiner also stated that the SIV/Rhesus model is not an "art accepted model for predictions of efficacy into the HIV/human model". However, the macaque model

used in the experiments described in the Declaration is accepted as an important animal model for infection of humans with HIV (see, e.g., Gardner, M.B., "Simian and Feline Immunodeficiency Viruses: Animal Lentivirus Models for Evaluation of AIDS Vaccines and Antiviral Agents" *Antiviral Res. 15*:267-286 (1991); Gardner, M.B., "SIV Infection of Macaques: a Model for AIDS Vaccine Development," *Dev. Biol. Stand. 72*:259-266 (1990); Johnson, P.R. and Hirsch, V.M., "SIV Infection of Macaques as a Model for AIDS Pathogenesis," *Int. Rev. Immunol. 8*:55-63 (1992); and McClure, H.M. *et al.*, "Nonhuman Primate Models for Evaluation of AIDS Therapy," *Ann. NY Acad. Sci. 616*:287-298 (1990)). For the Examiner's convenience, a Supplemental IDS citing these references which identify the macaque model as a valuable and appropriate model for human HIV infection, is being filed concurrently with this Amendment..

Rejection of Claims under 35 U.S.C. 112, second paragraph

The Examiner rejected Claims 46-49 and 71-72, stating that it was unclear how an antigen could "represent" different subgroups, different phases of infection, different tropisms or different routes of transmission. The claims have been amended to specify that the antigens are from different subgroups of the immunodeficiency virus; are from an immunodeficiency virus at different phases of infection; are from immunodeficiency viruses having different tissue tropisms; or from immunodeficiency viruses having different routes of transmission. For example, as described in the Specification, envelope proteins (Env) of HIV-1 undergoes a marked evolution in infected humans, and may vary, depending on the subgroup of the virus; the phase of infection; the tissue tropism of the virus; or even the route of transmission of the virus. The claims have been amended to reflect such possible alterations in an antigen.

Rejection of Claims under 35 U.S.C. 103

The Examiner rejected Claims 44, 51 and 81-89 as being unpatentable over Wolff *et al.* (U.S. Patent 5,693,622) or Felgner *et al.* (U.S. Patent 5,703,055), stating that it would have been obvious to use DNA encoding nef protein or gp120 protein for immunization against HIV.

Wolff *et al.* and Felgner *et al.* describe inoculation of mice with mRNA from the nef protein of HIV ("NEF mRNA"). As described in Example 9 (Column 28, particularly lines 23-31, of Wolff *et al.*, and Column 31, particularly lines 24-32), NEF mRNA was prepared, incorporated into a liposome preparation, and injected into the mice. Wolff *et al.* and Felgner *et al.* also describe inoculation of mice with DNA for the gp-120 gene (Example 19, at Column 36 of Wolff *et al.* and at Column 38 of Felgner *et al.*). Example 19 indicates that antibodies to the gp-120 protein were detected in the mice. Neither Wolff *et al.*, nor Felgner *et al.* describe immunization of a mammal by a DNA transcription unit whereby the mammal is protected from disease.

Obviousness is established only if the teachings of the cited art would suggest the claimed invention to one of ordinary skill in the art with a reasonable degree of certainty of successfully achieving the claimed results. One of ordinary skill in the art, given the teachings of Wolff *et al.* or Felgner *et al.* would not have been able to ascertain with a reasonable degree of certainty whether any immune response raised in the mice could have been effective in protection against disease. Mice do not develop AIDS upon being infected with HIV; therefore, the antibody response against gp-120 in mice could not have been used to test for protective immunization. Applicants have, for the first time, demonstrated protection against immunodeficiency disease in a recognized animal model by immunization with a DNA transcription unit.

CONCLUSION

In view of the amendments and the discussion presented above, the claims are in condition for allowance. Therefore, Applicant's Attorney respectfully requests that the Examiner reconsider and withdraw all the rejections.

If the Examiner feels that a telephone conversation would expedite prosecution, the Examiner is invited to call Elizabeth W. Mata at (915) 845-3558. If Elizabeth W. Mata cannot be reached, the Examiner is invited to call David E. Brook at (781) 861-6240.

Respectfully submitted,

David E. Brook Reg. No. 22,592

By for Elizabeth W. Mata

Elizabeth W. Mata
Registration No.: 38,236
Telephone (781) 861-6240
Facsimile (781) 861-9540

Dated: 5/24/99
Lexington, Massachusetts 02421